

# LymphoTrack® Low Positive Control and LymphoQuant® Internal Control for MiSeq® and Ion S5/PGM™ LymphoTrack Assays

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## Introduction

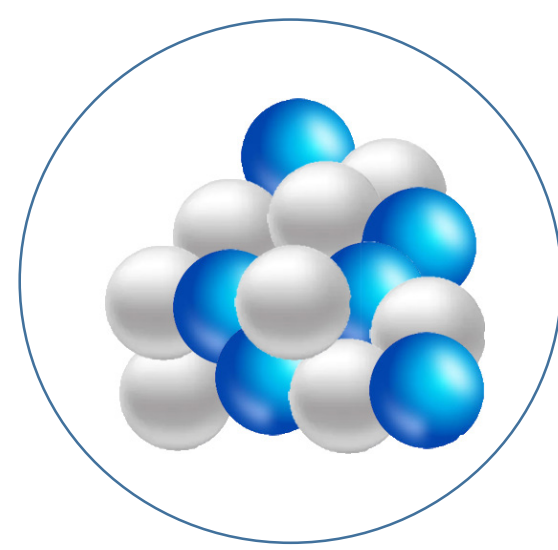
LymphoTrack® Assays with the associated LymphoTrack software have been developed for MiSeq® and Ion S5™ platforms. They are able to detect immunoglobulin (Ig) and T-cell receptor (TCR) clonal rearrangements in suspected lymphoproliferative disease (LPD) clinical specimens. The detected sample specific clonal V-(D)-J sequence in baseline specimens can be tracked in the follow-up samples to identify and monitor disease status. Typically, the subject-specific clonal rearrangements in follow-up specimens are present at very low levels ( $10^{-4}$  or below). When testing these specimens, it is important to include a low positive control (LPC) in the run and to report the estimated clonal cells in a sample. We report the design and development of these controls for LymphoTrack Assays: the LPCs run quality controls, and the LymphoQuant™ internal controls (LQIC) used for estimating clonal cells within a specimen.

## Materials and Methods

Two types of LPC and LQIC were developed, one for Ig (*IGHV* Leader, *IGH* FR1, *IGH* FR2, *IGH* FR3, and *IGK*), and one for TCR (*TRG* and *TRB*). Each LPC consists of clonal positive cell line DNA diluted in clonal negative DNA at  $\sim 10^{-4}$  level. Each LQIC is diluted clonal positive cell line DNA at a concentration of about 50 cell equivalent per  $\mu\text{L}$ . All LPCs and LQICs can be detected by the LymphoTrack Assays on either the Illumina MiSeq® or Thermo Fisher Ion S5/PGM™ platforms. Multiple lots of LPCs and LQICs were made and lot-to-lot variations were evaluated using contrived samples that were prepared by 10-fold serial dilution of clonal positive cell line DNA into clonal negative DNA at levels ranging from  $10^{-2}$  to  $10^{-5}$ .

## Principal of LymphoQuant Internal Control (LQIC)

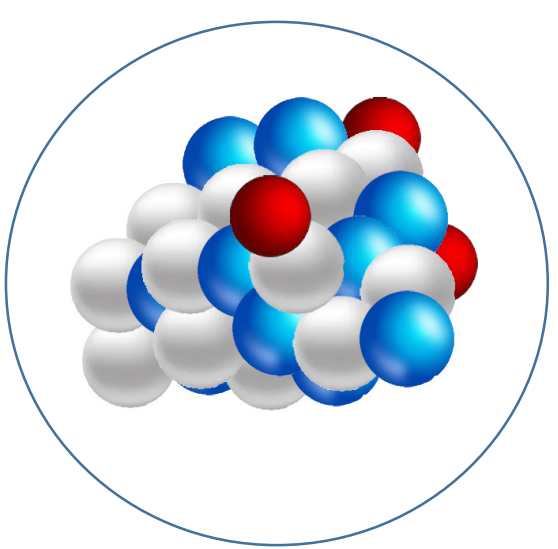
Sample without the presence of LQIC



Tested by LymphoTrack Assays

Sample output: % Total reads

Sample in the presence of LQIC



Tested by LymphoTrack Assays

Sample output: Clonal cell equivalents

- Non Clonal cells
- Clonal cells
- LymphoQuant

## Results: Intra and Inter Lot Variability for LQIC

Intra-Lot Mean and CV% of Frequency for LQIC Tested by Each LymphoTrack Assay

Lot	N	IGHV Leader		IGH FR1		IGH FR2		IGH FR3		IGK	
		Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%
1	12	6.16E-03	35.07	2.15E-03	28.12	7.18E-03	18.84	3.78E-03	55.54	4.40E-03	22.15
2	12	6.82E-03	8.56	2.10E-03	28.69	5.68E-03	14.54	3.56E-03	63.63	5.34E-03	23.79

Inter-Lot Mean and CV% of Frequency for LQIC Tested by Each LymphoTrack Assay

N	IGHV Leader		IGH FR1		IGH FR2		IGH FR3		IGK	
	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%
24	6.49E-03	24.4	2.12E-03	27.8	6.43E-03	20.8	3.67E-03	58.3	4.85E-03	24.7

## Results: Intra and Inter Lot Variability for LPC

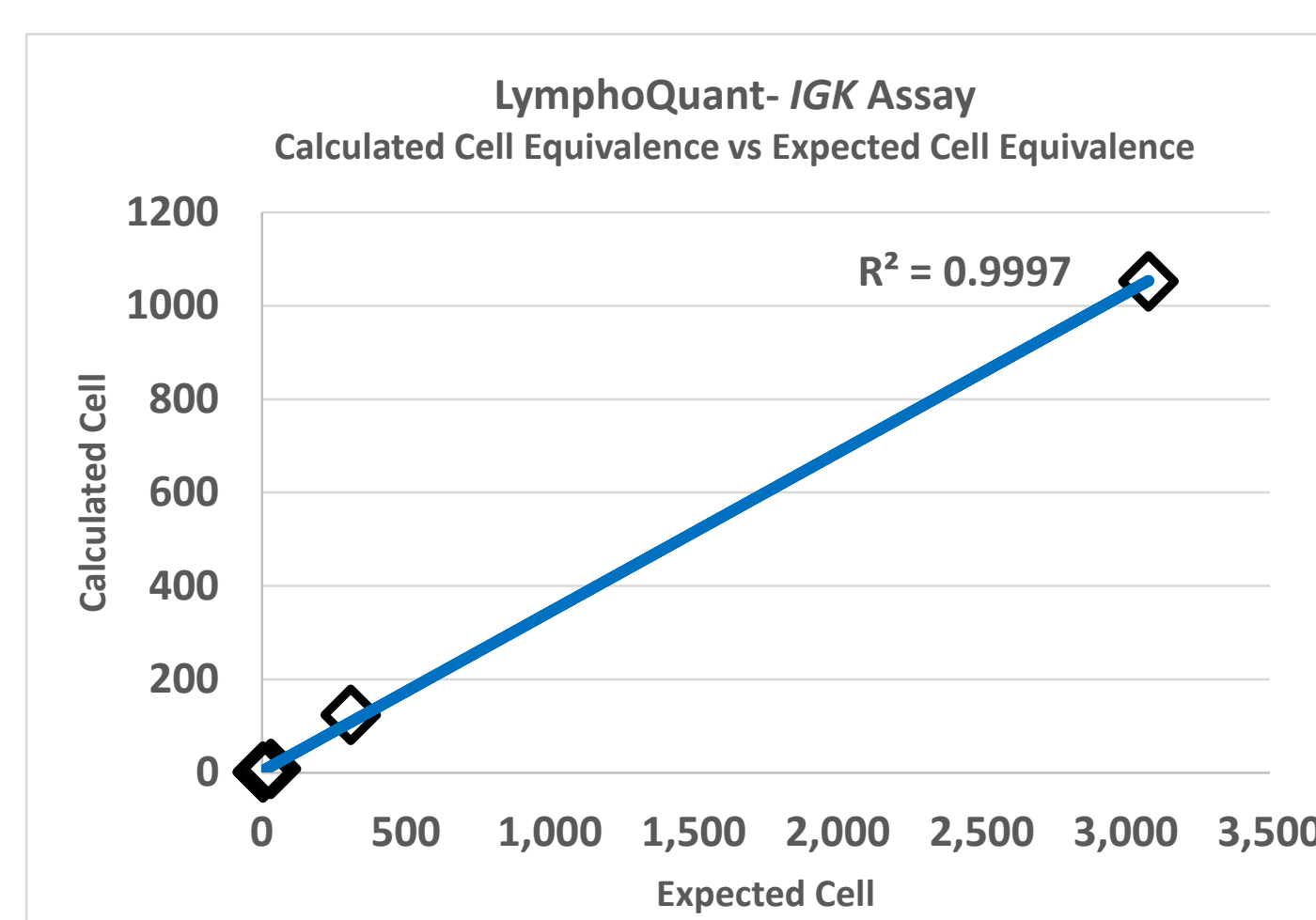
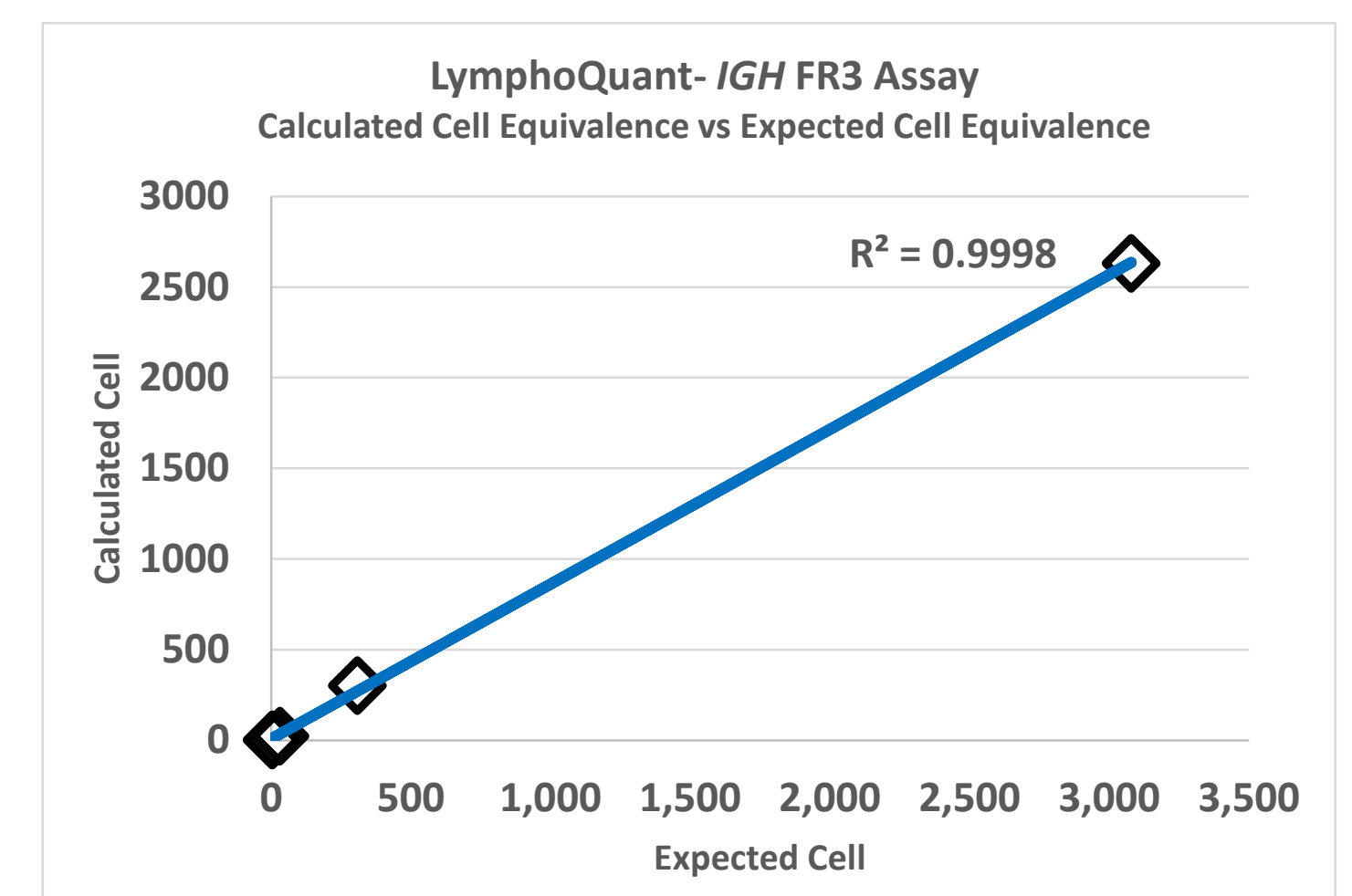
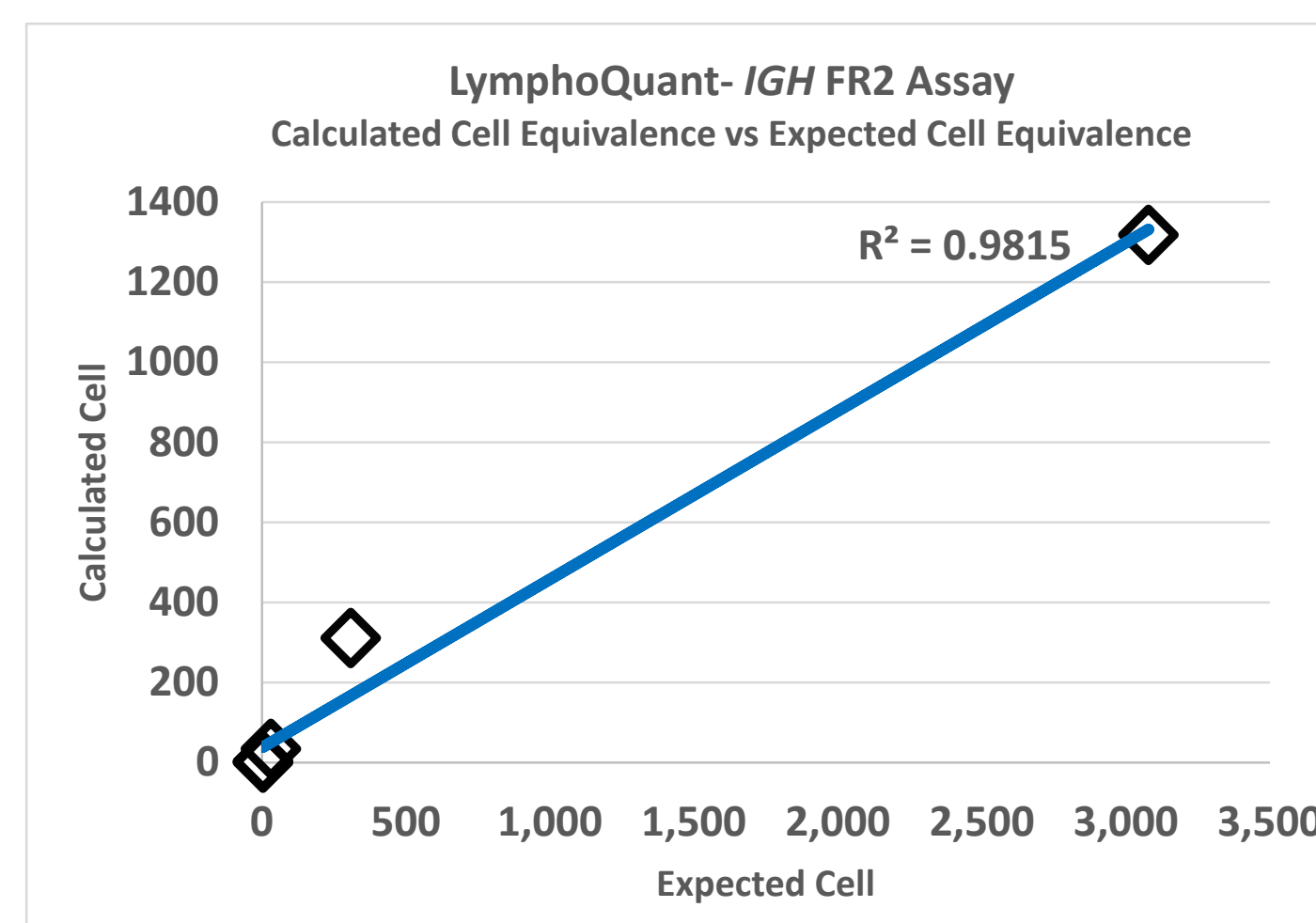
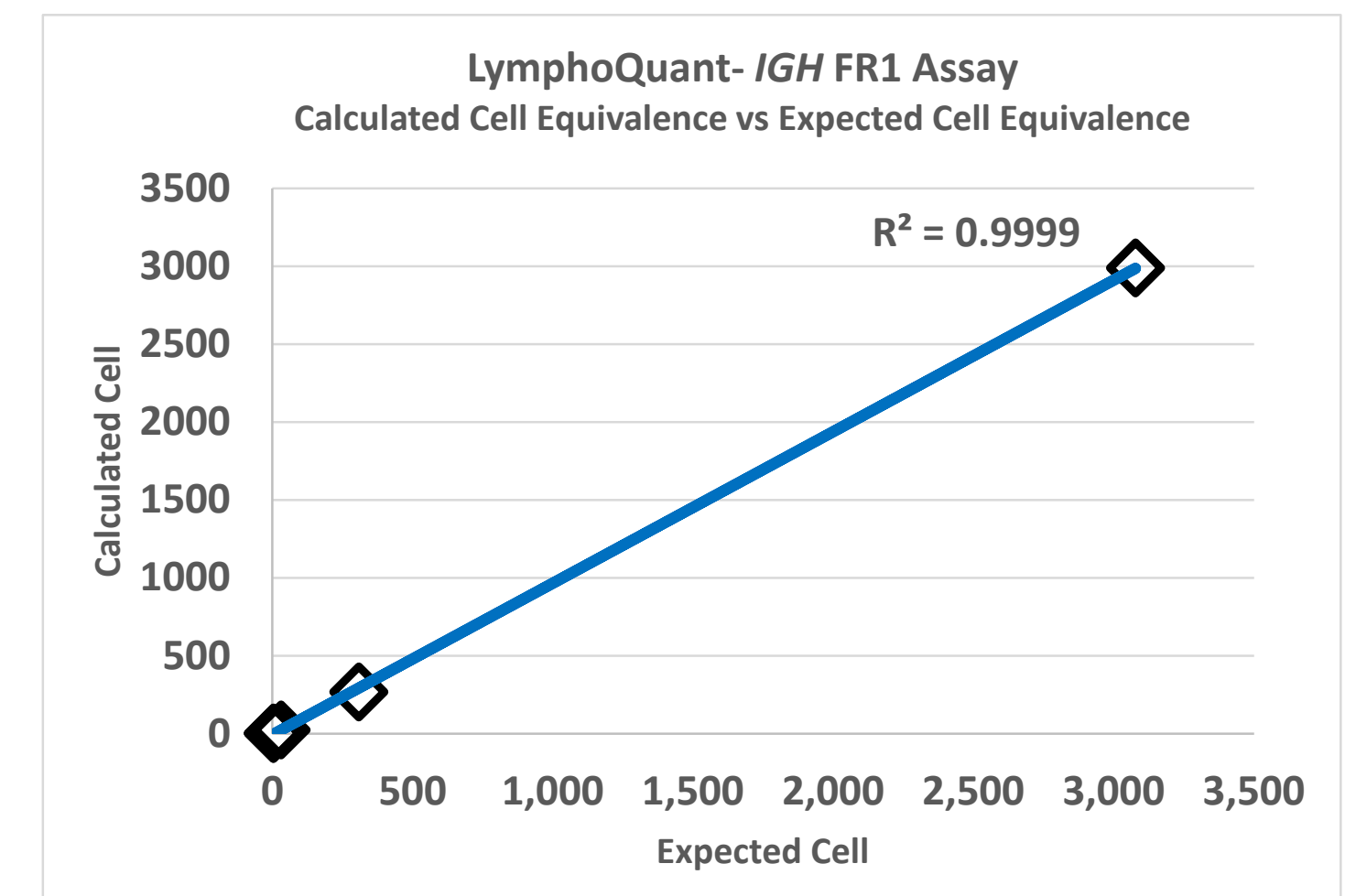
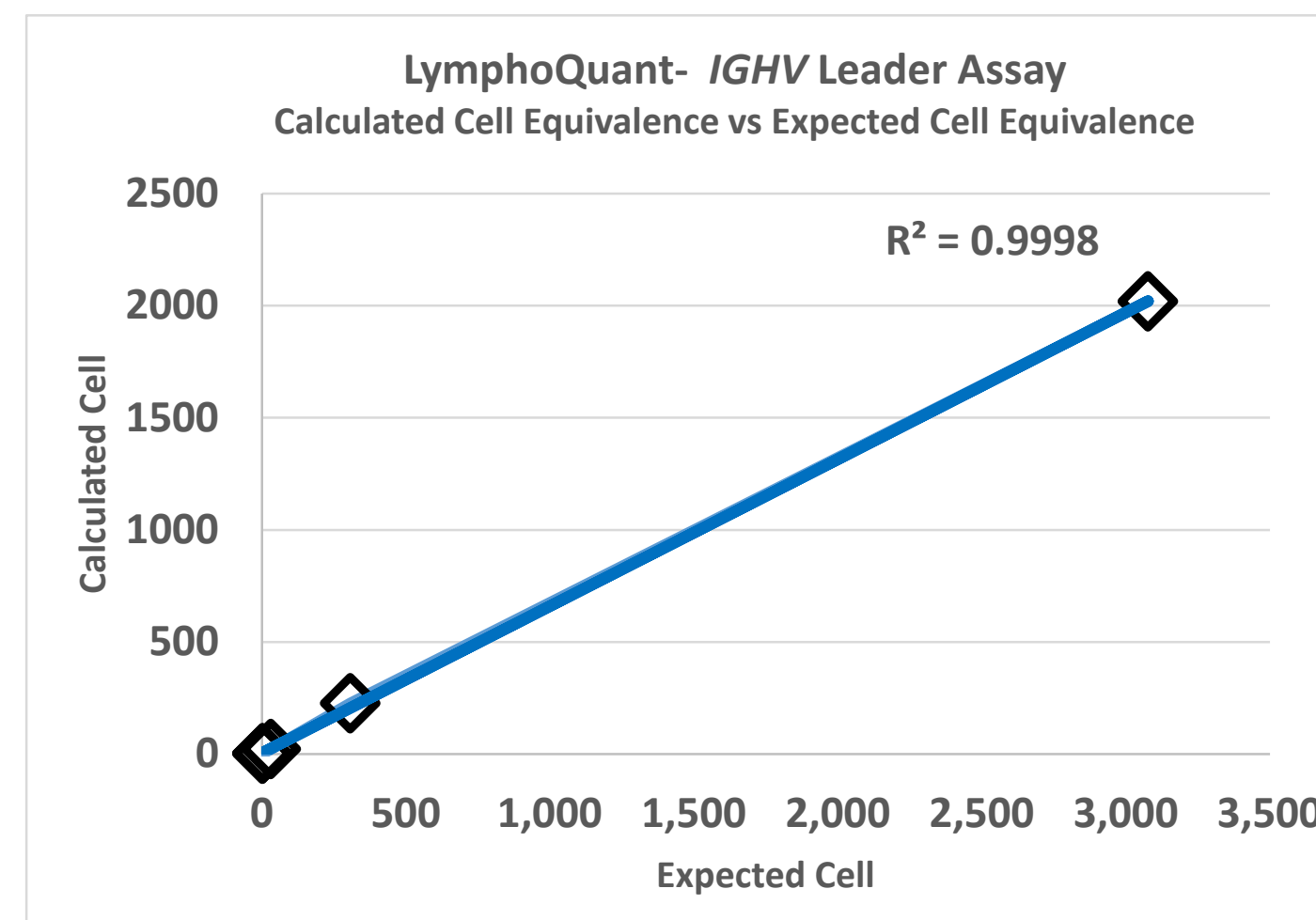
Intra-Lot Mean and CV% of Frequency for LPC Tested by Each LymphoTrack Assay

Lot	N	IGHV Leader		IGH FR1		IGH FR2		IGH FR3		IGK	
		Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%
1	12	1.99E-04	41.4	5.93E-05	66.8	2.61E-04	63.6	1.74E-04	65.5	8.53E-04	14.3
2	12	2.12E-04	44.1	5.73E-05	62.4	2.73E-04	35.7	1.33E-04	40.2	7.55E-04	20.3

Inter-Lot Mean and CV% of Frequency for LPC Tested by Each LymphoTrack Assay

N	IGHV Leader		IGH FR1		IGH FR2		IGH FR3		IGK	
	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%
24	2.06E-04	42.0	5.83E-05	63.5	2.67E-04	49.8	1.54E-04	58.1	8.06E-04	17.7

## Results: Calculated vs. Expected Cell Equiv. for Contrived Samples ( $10^{-2}$ to $10^{-5}$ )



Contrived Sample	Expected # of Clonal Cells
$10^{-2}$	3077
$10^{-3}$	308
$10^{-4}$	31
$10^{-5}$	3

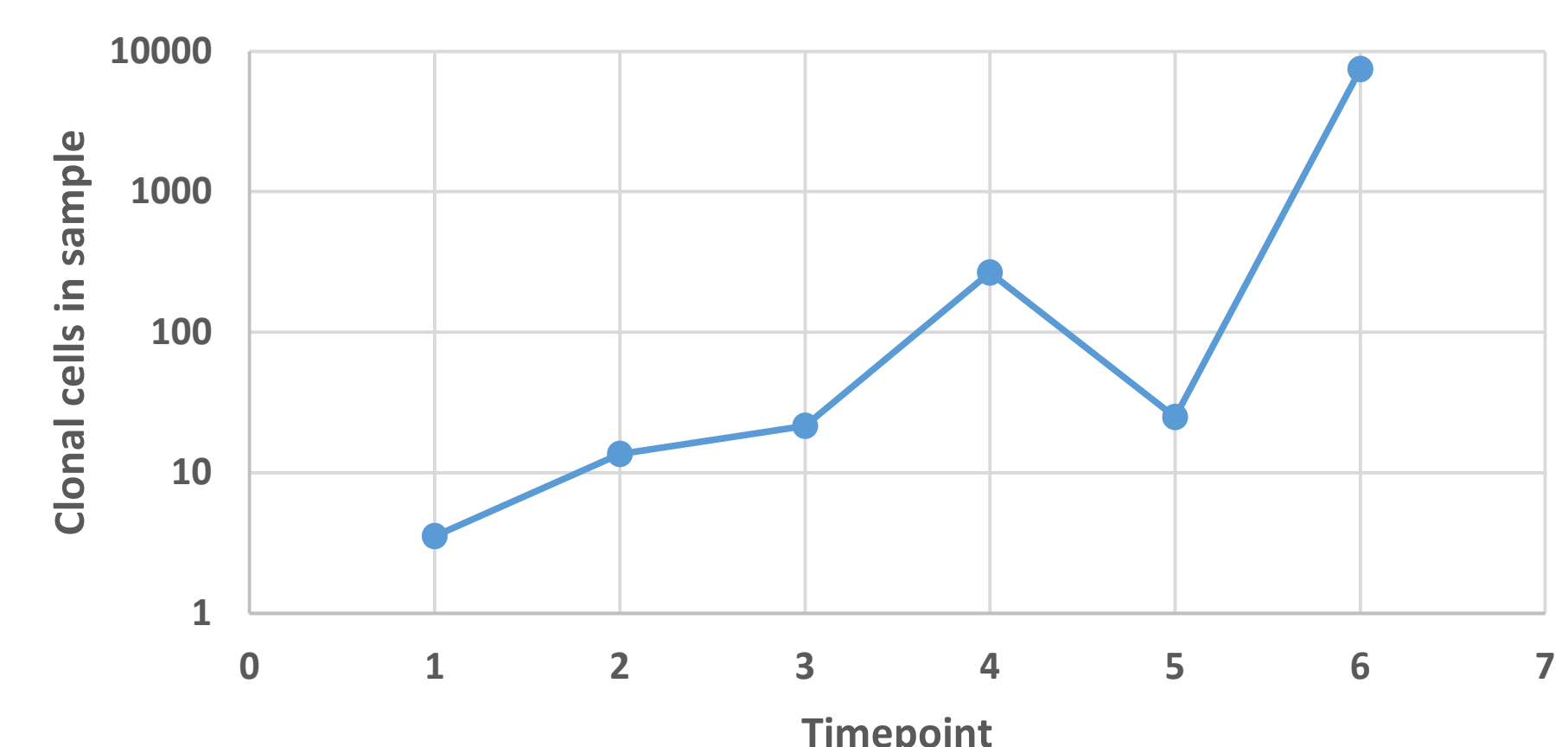
## Results: Comparison LQIC Results between MiSeq® and Ion S5™

LQIC Frequency Values Tested by LymphoTrack Assays on MiSeq® and S5 Platforms

Assay	MiSeq®		S5	
	Mean (n=12)	CV%	Mean (n=3)	CV%
<i>IGH</i> FR1	2.15E-03	28.12	6.47E-03	14.4
<i>IGH</i> FR2	7.18E-03	18.84	1.06E-02	11.4
<i>IGH</i> FR3	3.78E-03	55.54	4.64E-03	45.8

## Results: Calculated Clonal Cell Equivalence in Follow-Up Sample

Calculated Clonal Cell Equiv. in Follow-up Sample in the Presence of LQIC Tested by *IGH* FR3 Assay



## Conclusions

- We have developed two types of controls (LPC and LQIC) for LymphoTrack assays run on the MiSeq® and S5 sequencing platforms.
- Both inter- and intra-lot variability were assessed.
- The LQIC allows investigators to estimate clonal cells present in the specimen suggesting potential applications in detect and monitor minimal residual disease.